Mitigating the presence of *Alicyclobacillus acidoterrestris*, from beverage grade medium invert sugar syrup utilizing UV- irradiation: Part II

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abstract

Part I detailed the discovery and identification of *Alicyclobacillus acidoterrestris* in the incoming raw materials and processing system in our manufacturing facilities and the possible tainting with guaiacol, a metabolic product of this bacterium of our finished product used specifically in the beverage industry. Technical efforts applied as a result of this discovery leading to the adoption of a commercially available device and method to detect the presence of the TAB *A. acidoterrestris* bacterium is discussed. In Part II of this paper this method, in conjunction with conventional TAB plating techniques, were used to study the effect of low pressure UV lamp - irradiation on the presence of this bacterium in manufactured sugar syrup products on a pilot scale. Also examined and reported on were the effects of a commercial size medium pressure UV lamp system targeted for the same application.

Keywords: ??????

Introduction

Part I of this report described the discovery of *Alicyclobacillus acidoterrestris* presence in our refineries and products during 2003, and the spoilage related problems it can cause in a commercial beverage product due to production of trace amounts of guaiacol (see Wnukowski *et al.*, 2013). Also addressed were the technical efforts put forth in direct response to the issue and the related findings.

In 2008-2009 due to a change in the landscape of the sweetener marketplace, American Sugar Refining, (ASR) committed sizeable resources and capital to enable a number of their manufacturing facilities to produce and distribute Medium Invert Syrup (MIS). The MIS production targeted application in the beverage industry. Not soon after completion of one of these new MIS production systems located at the original TAB complaint involved refinery, it became apparent by customer specifications that the absence of guaiacol producing *Alicyclobacillus* would be required for their "hot fill" sensitive product line of beverages.

Based on the results described in Part I, it was decided that in order for ASR to compete for this segment of business, it would have to investigate any and all possible solutions for the elimination of guaiacol producing *A. acidoterrestris*. This paper deals with those efforts which included the use of both low and medium pressure UV lamp-irradiation for that purpose.

Materials and methods

As was the case and detailed in in Part I of this paper, in 2009-2010 three plating methods were employed confirm the presence of *Alicyclobacillus*. These studies were performed and/or recommended by either the customer's laboratory or a contract service laboratory. Thus exact details of the individual plating methods employed in this study will not be included (customer's methods propriety). However, general descriptions of the methods, media type, membrane versus pour plate, heat shock, growth period etc. will be listed to differentiate one from another. As documented in Part I, for detailed step by step method accounts please refer to Yakota, A., *et al*., 2007 pp. 64-68). Based on a unified methodology jointly designed by a consortium of ten beverage companies and the Japanese Fruit Juice Association, step by step standard methods for membrane filtration, pour plating and spreading for the detection of TAB are listed. The methods below, exceptions noted, followed these formats. Also detailed is a standardized direct detection method utilizing the peroxidase test for *A. acidoterrestris* confirmation.

Plating and enumeration methods employed in 2009-2010:

Method E - Supplied by Beverage Company (II) details included: 1) a 50 gram sample, 2) sample filtration through a 0.45 micron membrane, 3) heat shocked for 10 minutes at 80 +/- 2°C, 4) Pour plate using YSG agar (Difco) manufactured by Benton Dickerson company, Sparks, Maryland, and 5) incubate at 45 +/- 2°C for 5 days. If colonies are present a guaiacol producing organism differentiation test distributed by Cosmo Bio Co., Ltd. based on the peroxidase (method) conversion of guaiacol to tetraguaiacol was employed. This method was originally developed by (Niwa, M. *et al.*, 2003).

Method F – Supplied by Beverage Company (III) details included: 1) a 50 gram sample size, 2) sample added to YSG broth is heat shocked to 80 +/- 1°C for 10 minutes, 3) sample and broth are incubated at 44°C for 3 days, and 4) 0.1 ml of each sample spread

on YSG agar at pH 3.7 and incubated 44°C for 3 days. Guaiacol producing organisms are identified via peroxidase method (see Method E above).

The SMX 012A-250 SensiMedia *Alicyclobacillus* disposable microbial detection sensors were obtained from MicroBio Corporation, 5-7-10 Shimbashi, Minato-ku Tokyo 105-0004, Japan, www.Microbio.co.jp/. These devices were used to detect the presence of both *A. acidoterrestris* and *A. acidocaldarius* in our sucrose containing products.

Low Pressure UV Pilot Test Reactor - This single pass reactor constructed from 316L stainless steel was modified in terms of the placement of the mercury amalgam in order to compensate for the drop in UV radiation output performance for process/ sterilization of fluids in due to temperatures in excess of 43.3°C. A further description of the design particulars of the reactor are provided in Table 1.

Medium Pressure UV System used in Commercial Trials - The medium pressure UV system employed for the commercial scale trials was manufactured by Aquionics Inc., 21 Kenton Lands Road, Erlanger, KY 41018. A brief description of the system appears in Table 2.

Table 1. UV pilot test reactor details

Parameter	Value
Lamp type	Low pressure high output amalgam
Lamp wattage	190 W
UV output total watts [1]	68 W
Tube diameter	15 mm
Quartz sleeve diameter	23 mm
Reactor length	58”
Reactor inner diameter	1.5”

Table 2. Commercial scale medium pressure UV system details

Acquionics Photon Disinfection Unit Model # PMS150P4/4A	
Design flow	200 gpm
System sizing based on %UVT @254nm	40%, 10 mm path
Lamp type	4-2.5 kW medium pressure lamps
Power consumption per lamp	2.1 kW
End of lamp life UV dose	>30 mJ/cm^2
Expected lamp life	4000-8000 hours
UV chamber construction	316L stainless steel
Operating pressure	100 psi
Working fluid temperature	40-167°F

Results and discussions

Due to the familiarity and experience of our refineries, filtration was considered first as possible answer to the TAB presence. Although most of our refineries make use of some mechanical filtration in its operations, micro or membrane filtration would not be an option due to the high percent solids concentration and the accompanying viscosity. Most of the existing processing is accomplished with pressure leaf filtration which is not nearly effective in the removal of microorganisms. An attempt was made to increase the effectiveness of one of the filters in the sugar syrup operation by moving to the use of a tighter porosity filter aid as a per-coat. A > 0.5 kg. /m^2 precoat of Standard Super-Cel was substituted for the currently used Cleatom FW50. Based on specification comparisons for the two products for median particle size in microns (42.0 versus 15.4) and Permeability (3.5 versus 0.28 d’Arcys), the Super-Cel product was a significantly tighter filtering product. In fact, in a laboratory bench scale experiment *A. acidoterrestris*, as identified by the SMX 012A-250 tools, was removed by filtration through a laboratory filter pre-coated with 0.7 kg. /m^2 Standard Super Cel. However, in the industrial scale experiment all samples taken from either a control press pre-coated with the FW50 or the test press pre-coated with Standard Super Cell were positive for the presence of *A. acidoterrestris* (see Table 3).

As a result of these tests, the need to investigate the use of UV disinfection in terms of this application became evident rather quickly.

A. *Alicyclobacillus* - low pressure UV pilot study

Our use of Ultraviolet (UV) Sterilization, as a whole, was not new. For many years, a number of our facilities have used medium pressure lamp UV disinfection as a supplemental means to the plants’ primary processes used to control typical microbiological populations such as common mold, yeast and vegetative bacteria. However, because of this experience, our organization was also knowledgeable in regard to the fact that the amount of UV energy required to kill microorganisms varies from one organism to another. Furthermore, endospore forming organisms, such as TAB in this case, are much more resistant to UV radiation than others.

Attempts of finding any technical literature references as to any direct UV dosage studies conducted on *Alicyclobacillus* were not too successful. Yakota, A., *et al.*, (2007) cites that the reported

Table 3. Effect of filtration through Standard Super-Cel filter aid on the removal of *A. acidoterrestris*

Sample	Time	Tab plate count cfu/50 grams **(Method F.)**	*A. acidoterrestris* SMX 012A-250
Feed syrup	10:00 AM	78	positive
Effluent FW50 filter press	10:00 AM	99	positive
Effluent Standard Super-Cel filter press	10:00 AM	61	positive
Feed syrup	2:00 PM	120	positive
Effluent FW50 filter press	2:00 PM	130	positive
Effluent Standard Super-Cel filter press	2:00 PM	277	positive

Table 4. % UVT of Medium Invert Syrup (MIS) at different temperatures

MIS temperature °C	% UV transmission @ 253.7 nm, 1 cm cell
25	3.4
51	1.3
57	0.8
73	0.4

Table 5. % UVT measurements on selected sugar and Medium Invert Syrups

Sample	%UVT @ 253.7 nm, 1 cm cell, ambient temp.
67.7% solids sugar syrup	55.0
73.7% solids sugar syrup feed to MIS reactor	32.7 – 40.1
73.7 % solids Medium Invert Syrup	2.1
77.1 % solids Medium Invert Syrup	2.2

D or D10 value (the UV radiation required to achieve 90% reduction of a specific microorganism) for a juice drink isolate of *A. acidocaldarius* AC-1 spores for UV radiation was 150 µW. min/cm2 (9.0 mJ/cm^2). Torkamani and Niakousari (2011) determined that a UV dose of 125 mJ/cm^2 was needed in order to reduce the overall aerobic plate count in reconstituted and fresh of orange juice by approximately 97%.

With little information readily available, it was decided to start our own program and both low and medium lamp based UV sterilization supplying companies were contacted. In each case although there was some familiarity with our products due to the sugar syrup applications, the percent UV Transmittance (%UVT) measured at 253.7 nm wavelength for the MIS product to be treated was requested. The results of these measurements are listed in Table 4.

It is well known that the more ultra violet light that is absorbed by the fluid being irradiated the less light energy will be available to inactivate the microorganisms present (http://www.emperoraquatics.com/whatisuvsterilization, 2011). These %UVT values along with the projected process flow of 379-454 liters per minute (LPM) and the relative amount of UV dose that would be needed to address this endo-spore forming bacteria, led the majority of UV suppliers to be hesitant to take on this application. This prompted reconsideration on where the product sterilization needed to take place. As a result some further %UVT measurements were made (see Table 5).

The new %UVT values recorded for the saturated sugar syrup feed to the MIS reactors attracted some mild interest from both low and medium pressure UV manufacturers. However, this newly proposed treatment point was not without its own problems. Due to the nature of the MIS manufacturing process and the fact that the syrup is supersaturated in respect to sucrose at ambient conditions, the temperature of this syrup is typically in the area of 72-78°C during manufacture. The UV spectrum in terms of disinfection can be divided in to four distinct spectral regions: Vacuum UV (100-200nm), UV-C (200-280 nm), UV-B (280-315 nm), and UV-A (315-400nm). Of the four, UV-C is identified as the most lethal range of wavelengths for microorganisms. As a result, low pressure lamps which produce virtually all of their UV output at a wavelength of 254 nm are cited as being superior in producing light of germicidal effectiveness as opposed to medium pressure lamps which produce a relatively broad band of wavelengths, with many of them outside the UV-C region (http://www.uvcomparison.com/uvscienc.php, 2011).

Unfortunately low pressure lamps are known to lose their output rapidly if the temperature of the fluid rises above an optimum operating temperature of approximately 41°C.

In order to address the relatively high temperature of the process stream, low pressure UV lamp system manufacturer modified the placement of the amalgam in order to compensate for the sterilization of fluids in excess of 71°C (refer to Materials and methods, Table 2 for details of the pilot reactor design). This offer, coupled with the lack of availability and ability to obtain any comparable medium based UV pilot system at that time, led to this design being evaluated initially.

Having agreed upon the UV system to be evaluated, the only remaining criteria to be agreed upon by our technical staff was what method would be used to assess its success or failure in eliminating the guaiacol producing TAB presence in our finished product. It was decided to employ a variation of the method used by the company we were seeking to business with, namely Beverage Company (III), (Method F) for enumeration and isolation of the bacteria. The major modification was the use of 0.45 membrane filtration of the sample which was transferred to acid YSG medium for growth. However, because of some of the discrepancies already recorded in results, what would be used to identify the presence of spoilage capable *Alicyclobacillus* (guaiacol producing) was uncertain. This was due to our consistent findings, where the SensiMedia SMX 012A-250 tools would typically yield positive for the presence of *A. acidoterrestris* in refinery #1 sucrose sugar syrups, while the Method F means of identifying guaiacol producing TAB, via peroxidase method, was typically negative.

In order to try and make sense of this contradiction the manufacturer of the SMX 012A-250 tool was asked for their input on the problem. At their request, a sample of syrup was sent to MicroBio's attention. This sample was characterized as having been plated numerous times by Method F, yielding negative results for the peroxidase guaiacol confirmation test and yet consistently testing positive for *A. acidoterrestris* using the SMX 012A-250 tool. The results of their investigation were that they clearly observed low levels of *Alicyclobacillus* bacteria including *A. acidoterrestris* present in the sugar sample, which was confirmed by both their sensor and the peroxidase method. Additionally, MicroBio commented that because of these low levels Method F would have difficulty at times detecting *A. acidoterrestris* based on the low levels present and probability alone. MicroBio's observations as to relatively low levels of TAB bacteria present are supported with the plating results reported in both parts of this paper. For example < 10 cfu per gram detected for finished products (i.e. Part I, Tables: 3, 5, and 7) and < 20 cfu per gram on average reported in Tables 1, 3, 6 and 9 appearing in this portion of the report. Some details of their investigation are illustrated in Figure 1.

Figure 1. MicroBio's investigation of syrup sample testing negative for *A. acidoterrestris* originally utilizing Method F.

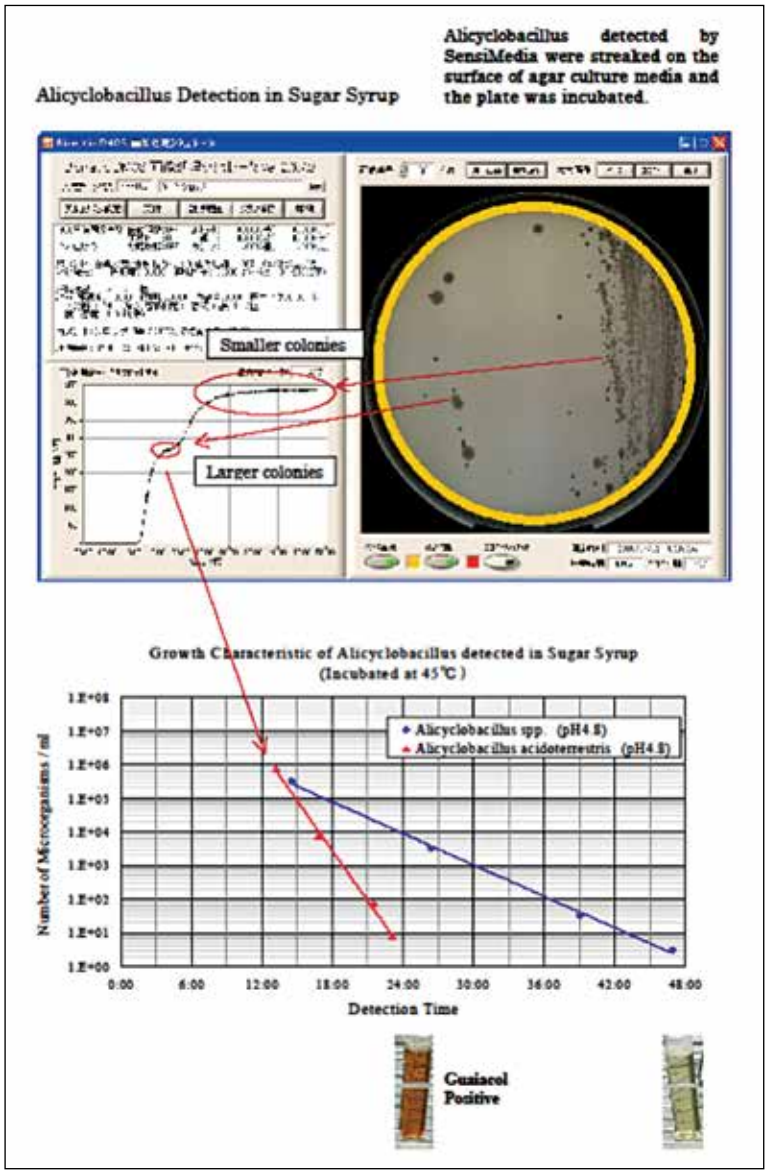

Figure 2. Photographs illustrating the installation of the low pressure lamp UV pilot reactor in the Medium Invert Syrup production system

Note that two distinct *Alicyclobacillus* species were isolated by MicroBio, *A. acidoterrestris* and another labeled as *Alicyclobacillus* spp. These two species were identified by their individual growth characteristic monitored precisely in real time utilizing their original digital microscope technology. While incubating standard plates under test, it monitors the plates automatically at the microscopic level at the time interval set selected. It detects the presence of microorganisms rapidly and counts colonies precisely, presenting a colony-count graph at the same time.

Since 2003 numerous species of *Alicyclobacillus*, other than acidoterrestris, have been associated with or thought to have promoted spoilage through guaiacol production, for example, *A. acidiphilus, A. herbarius*, and *A. pomorum* (Yakota, A., *et al.*, 2007, Duvenage, W., 2006). However, overall these citings have been in the minority as compared to spoilage cases associated with *A. acidoterrestris*. As a result of this and the investigation of MicroBio, our Technical Department decided that the measurement tools utilized to determine the effectiveness of the low pressure UV system would be plating with acidified YSG gar (Method F) and confirmation of the presence or absence of *A. acidoterrestris* by use of the SMX 012A-250 tools.

The pilot low pressure UV lamp reactor (detailed in Table 1 Materials and methods) was installed by connection to a separate auxiliary line off the MIS pump which moves saturated sugar syrup at approximately 74-75 brix from the dissolution tank to the reactors for conversion to medium invert syrup. The flow rate delivered to the reactor was controlled by a series of hand valves. A picture of the installed low pressure lamp UV pilot reactor appears in Figure 2.

The initial flow rate for the first trial of the low pressure UV reactors was approximately 7.6 LPM. The average feed temperature to the reactors was 70°C. Samples were taken in to and out of the reactors every ten minutes for 60 minutes. The samples were tested for TAB plate counts as well as *A. acidoterrestris* using SensiMedia. The results are detailed in Table 6.

This first low pressure UV trial's results were quite encouraging, indicating an average of 89.9% reduction in TAB colony forming units or a so called D10 value or 1-log elimination of the bacteria. No effects to the sensory characteristics of the syrup, flavor, and color were detected as a result of the UV irradiation. Additionally, all SensiMedia screening for the presence of *A. acidoterrestris* were positive for the syrup samples taken prior to entering the reactor and negative for the effluent syrup sample.

Subsequent pilot tests were staged in which the above conditions were duplicated as well as others conducted at varying flow rates or UV dose rates in the reactor. These syrup

Table 6. *Alicyclobacillus* results for first low pressure UV reactor trial, syrup Brix 74-75, average flow. Rate 7.6 LPM, average temperature 70°C

Sample, Time, min.	Feed syrup to reactor		Effluent syrup from reactor	
	TAB count, cfu/10g	*A. acidoterrestris* SMX 012A-250	TAB count, cfu/10g	*A. acidoterrestris* SMX 012A-250
1	60	positive > 1cfu/50g	17	negative < 1cfu/50g
10	68	positive > 1cfu/50g	5	negative < 1cfu/50g
20	100	positive > 1cfu/50g	13	negative < 1cfu/50g
30	85	positive > 1cfu/50g	3	negative < 1cfu/50g
40	76	positive > 1cfu/50g	5	negative < 1cfu/50g
50	107	positive > 1cfu/50g	5	negative < 1cfu/50g
60	128	positive > 1cfu/50g	12	negative < 1cfu/50g
Average count	89	-	9	-

flows varied from 3.0 to 22.7 LPM. The results of selected trails appear in Table 7.

The viscosities of the feed syrup at these temperatures were in the area of 40-50 centipoises and the recorded pressure drops across the reactor ranged from 2 to 20 psi for the flow rates employed. The overall results of the trials were again favorable. As expected, the *Alicyclobacillus* colony kill decreased with the decrease in UV dose, and increase in syrup flow. No presence of taint producing A. acidoterrrestris was detected for any samples utilizing the SensiMedia tests; even at the highest employed 22.7 LPM flow rate, or lowest UV dose rate. However, two positive test results were recorded for effluent samples collected during one of the 7.6 LPM trials via the peroxidase test confirmation. Unfortunately, repeat testing on these samples could not be conducted due to insufficient amounts of sample collected.

Detailed values as to the calculated UV dose supplied by this low pressure amalgam lamp pilot reactor at varying flows of water were requested from the manufacturer. The data was received and appears in Table 8. Comparing the syrup flow rates used in the UV pilot tests with these calculated UV water dose, the equivalent clean water UV doses can be estimated for the pilot reactor syrup flows. These values along with the average colony counts and percent reduction in TAB organisms appear In Table 9.

Plotting these UV dose rates versus the average sugar syrup % *Alicyclobacillus* colony reductions for the same flow rates yields a curve from which the D10 UV dose (neglecting the %UVT of the syrup) can be estimated (refer to Figure 3). According to the graph, the approximate UV dose value (based on clean water) needed to achieve a 90% or 1-log reduction in the TAB colonies is 315 mJ/cm^2.

Next investigated was the observation of the residual *Alicyclobacillus* colony counts even after the application of the highest UV doses (lowest reactor syrup flow rates). These counts were still recorded while the SensiMedia tests had indicated to the level of their test detection (1cfu/50g) that no *A. acidoterrestris* was detected. A limited test was performed where some previously irradiated and screened samples were tested again not only for *A. acidoterrestris* but also for *A. acidocaldarius* with the SensiMedia devices (see Table 10). As was expected, three out of the four samples tested (including one after UV dosing) were found positive for the presence of *A. acidocaldarius*. These results were consistent with our findings and testing in 2003-2004 that indicated that *A. acidocaldarius* was the more prevalent

TAB species typically found and associated with our sugar products and is not typically equated with spoilage due to guaiacol production. Also of note, is that a seeming reduction in the overall count of *A. acidocaldarius*, due to UV irradiation, was also confirmed due to the negative test result achieved for the effluent syrup sample from the pilot reactor.

The final piece of work conducted in association with these low pressures UV pilots trials was an attenpt to furher substantiate our results in terms of confiming the reducticon/elimination of the *A. acidoterrestris* species in our sugar samples as determined by use of the MicoBio tools. In order to do this, again a limited test utilizing a contract labortatory employing genotypic microbial identificatins services was utilized. Comparative DNA sequencing of the 16S rRNA gene in bacteria has been proven and is the most accurate and reporducible method for identifying unknown organisms at the speies level ((Yakota, A., *et al.*, 2007 pp83).

Arrangements were made to take two (2) larger than usual sugar samples samples, entering and leaving, the UV pilot reactor during one of the 7.6 LPM flow rate trials. These samples were submitted to our typical confirmation checks for acidoterrestris employing the SMX 012A-250 tools. As usual, the sample prior to entering the reactor was positive and the effluent negative. Next, two attempts at plating each of these samples were performed in accordance with the conditions laid out in Method F.

One plate preparation method involved taking a 50 gram sample in to a sterile acidified YSG broth, heat shocking the broth at 80o for ten minutes, cooling the sample to below 50o, and incubating the broth at 44°C for 3 days. At the end of that time, 0.1 ml of the sample was spread on YSG agar and incubated for an additional 3 days at 44°C. These plated samples, before and after UV reactor dose, were identified as “Broth”. The second plating attempt involved heat shocking the sample at 80°C for ten minutes cooling the sample to below 50°C and filtering 10 grams of the sample through a 0.45 micron membrane. The membrane was placed on an YSG agar plate and incubated at 44°C for 3 days. These samples were identified as “SM” before and after. All four plates were sent to the contract testing laboratory for all viable colony identification via 16s rDNA sequencing.

The organism identified in both the “Broth” and “SM” plates for the sample prior to UV dosing was *A. acidoterrestris*. In the cases for both samples representing plating of the sugar syrup after UV irradiation, the broth sample exhibited no TAB growth,

Table 7. Summary of low pressure UV reactor trials at different sugar syrup reactor flow/dose. Rates for 74 -75 Brix sugar syrup

Sample Time (min.)	Feed syrup to reactor				Effluent syrup from reactor			
	Flow rate LPM	TAB count cfu/10g	*A. acidoterrestris* SMX 012A-250	*Confirmation Guaiacol producing	Temp. °C	TAB count cfu/10g	*A. acidoterrestris* SMX 012A-250	*Confirmation Guaiacol producing
5	3.0	160	Positive > 1cfu/50g	Negative	76	3	Negative < 1cfu/50g	Negative
10	3.0	139	Positive > 1cfu/50g	Negative	75	0	Negative < 1cfu/50g	Negative
15	3.0	240	Positive > 1cfu/50g	Negative	75	5	Negative < 1cfu/50g	Negative
20	3.0	111	Positive > 1cfu/50g	Negative	77	3	Negative < 1cfu/50g	Negative
25	3.0	68	Positive > 1cfu/50g	Negative	77	5	Negative < 1cfu/50g	Negative
30	3.0	59	Positive > 1cfu/50g	Negative	77	10	Negative < 1cfu/50g	Negative
5	9.5	67	Positive > 1cfu/50g	Negative	73	0	Negative < 1cfu/50g	Negative
10	8.7	4	Positive > 1cfu/50g	Negative	74	3	Negative < 1cfu/50g	Negative
15	8.7	27	Positive > 1cfu/50g	Negative	74	12	Negative < 1cfu/50g	Negative
20	8.7	70	Positive > 1cfu/50g	Negative	74	40	Negative < 1cfu/50g	Positive
25	8.7	51	Positive > 1cfu/50g	Negative	74	1	Negative < 1cfu/50g	Positive
30	10.6	182	Positive > 1cfu/50g	Negative	74	35	Negative < 1cfu/50g	Negative
35	7.6	74	Positive > 1cfu/50g	Negative	74	4	Negative < 1cfu/50g	Negative
40	7.2	83	Positive > 1cfu/50g	Negative	73	47	Negative < 1cfu/50g	Negative
45	7.2	101	Positive > 1cfu/50g	Negative	73	42	Negative < 1cfu/50g	Negative
1	15.1	63	Positive > 1cfu/50g	Negative	76	5	Negative < 1cfu/50g	Negative
5	15.1	58	Negative > 1cfu/50g	Negative	76	71	Negative < 1cfu/50g	Negative
10	15.1	82	Positive > 1cfu/50g	Negative	76	45	Negative < 1cfu/50g	Negative
15	22.7	110	Positive > 1cfu/50g	Negative	76	27	Negative < 1cfu/50g	Negative
20	22.7	88	Negative > 1cfu/50g	Negative	76	30	Negative < 1cfu/50g	Negative
20	22.7	99	Positive > 1cfu/50g	Negative	76	13	Negative < 1cfu/50g	Negative
30	22.7	71	Positive > 1cfu/50g	Negative	76	89	Negative < 1cfu/50g	Negative

*Results obtained utilizing the peroxidase confirmation procedure detailed in Methods E. & F.

and the SM sample failed to produce any high quality sequence data needed to generate an identification report.

B. *Alicyclobacillus* – medium pressure UV tests

Although the pilots trials effectively demonstrated the potential for low pressure UV lamp irradiation to reduce the presence of taint producing TAB colonies in our Medium Invert Syrup production system, there were still issues to be considered especially on a commercial scale. The current pilot unit supplier had altered the amalgam placement for the trial, but currently employed commercial low pressure high output UV systems

Table 8. Calculated clean water UV dose at reactor chamber

Water flow rate	Calculated UV dose
3.8 LPM	436 mJ/cm^2
7.6 LPM	218 mJ/cm^2
11.4 LPM	145 mJ/cm^2

Table 9. UV dose and percent TAB reduction for sugar syrup low pressures UV pilot trials

Average Sugar Syrup Flow Rate, LPM	Average Influent TAB cfu/10 g	Average Effluent TAB cfu/10 g	Average % Reduction	UV Dose mJ/cm^2
3.0	130	4	97	545.0
8.7	73	9	88	189.6
15.1	68	40	40	108.8
22.7	92	40	57	72.5

are characterized by outputs that decline with significantly higher temperatures. This is why they are typically confined to applications <43.3°C. There were also concerns that the lamp life could be considerably shortened by our projected operating temperatures range of 71-77°C Finally, at the less than low dose requirements found in our investigations the low pressure system would be quite large, probably consisting of multiple reactors in series or parallel.

As a result, we continued to be interested in the evaluation of the more familiar medium pressure system for this application which would not be subject to the same lamp output declines at the high process temperatures. This initiative was also not without problems. Any medium pressure UV system commercially installed and operating on sugar syrup production at any of our facilities was subject to the some of the findings described in this paper. Namely, that due to the type of the sugar syrup processing employed at the other refinery locations, the dominant *Alicyclobacillus* species present was not *A. acidoterrestris* but *A. acidocaldarius*. Likewise the availability, scheduling, and resources available were not conducive into beginning any medium pressure pilot trial at the problematic refinery. This culminated in the end into investigating the performance of a commercial medium pressures UV system placed at an affiliated liquid sugar melting station. This facility, because of the nature of their business, consistently produced sugar syrup from refined sugar from multiple origins and plants. If any location might be privy to sugar containing a measurable *A. acidoterrestris* population, this was the best chance for success.

Figure 3. Graph illustrating % tab colony reductions versus calculated clean water UV doses for the low pressure pilot reactor

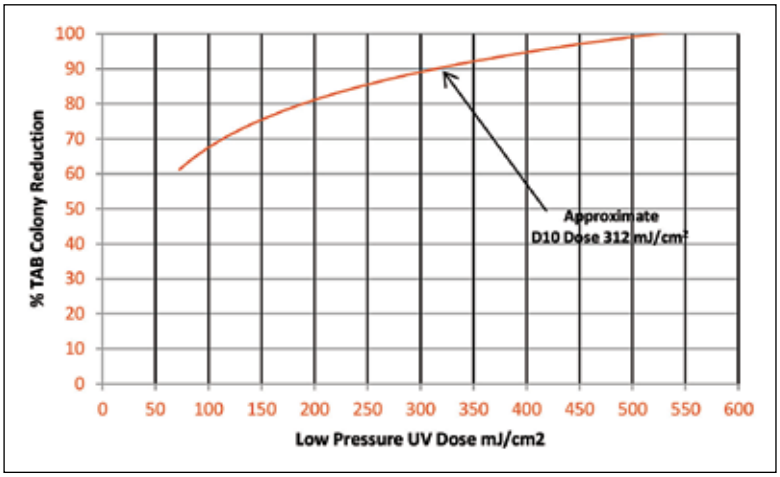

Table 10. SensiMedia test results for *A. acidoterrestris* and *A. acidocaldarius* on samples of sugar syrup before and after the UV pilot reactor

Sample	Trial flow rate, LPM	*A. acidoterrestris* SMX 012A-250	*A. acidoterrestris* SMX 012A-250
Sugar syup before UV reactor	8.7	Positive	Positive
Sugar syup after UV reactor	8.7	Negative	Negative
Sugar syup before UV reactor	15.1	Positive	Positive
Sugar syup after UV reactor	15.1	Negative	Positive

The medium pressure UV system, placed at the melt station, was manufactured by Aquionics. The specifics of the system are listed in Table 2. in the Materials and methods section of the paper. Unfortunately, the only dose information obtainable was a standard manufacturer end of lamp life dose specification of > 30 mJ/cm2 for influent syrup at approximately 43.3°C, 67-68 Brix (% solids), 757 LPM feed rate and a %UVT of approximately 40.

This investigation was initiated by the routine receipt of samples from the melt station representing syrup samples before and after irradiation by the unit. This practice was repeated for a number of sugar shipments converted into syrup from different origins. As expected, the vast majority of the samples screened with the SMX 012A-250 tools yielded negative results for the presence of *A. acidoterrestris*, consistent with our findings for syrup produced from crystallized commercial sugar. However, finally with the receipt of a set of samples processed from a sugar, Mexican in origin, some positive results for the presence of *A. acidoterrestris* were obtained. All of the effluent UV irradiated samples obtained for this set of samples were negative.

In order to at least supplement these initial findings, it was requested that the melt station continue to send only samples associated with the processing of sugar from the same origin. Within the timeframe associated with the scope of this paper this translated in to two additional sets of samples received and screened. The results for all three of the medium pressure UV lamp trials appear in Table 11.

Overall the medium pressure UV results for the

Table 11. *A. acidoterrestris* result summary for medium pressure UV commercial reactor trials, syrup flow rate 200 gpm, temperature approximately 100°F, syrup Brix 67-68

Sample identification	Feed syrup to reactor *A. acidoterrestris* SMX 012A-250 result	Effluent syrup from reactor *A. acidoterrestris* SMX 012A-250 result
Trial I Sample Set #1	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial I Sample Set #2	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial I Sample Set #3	Negative < 1cfu/50g	Negative < 1cfu/50g
Trial I Sample Set #4	Negative < 1cfu/50g	Negative < 1cfu/50g
Trial I Sample Set #5	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial I Sample Set #6	Negative < 1cfu/50g	Negative > 1cfu/50g
Trial II Sample Set #1	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #2	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #3	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #4	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #5	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #6	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #7	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #8	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #9	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #10	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial II Sample Set #11	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #1	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #2	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #3	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #4	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #5	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #6	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #7	Positive > 1cfu/50g	Negative < 1cfu/50g
Trial III Sample Set #8	Positive > 1cfu/50g	Positive > 1cfu/50g
Trial III Sample Set #9	Positive > 1cfu/50g	Positive > 1cfu/50g

commercial system described, sans the last two effluent samples received for Trial III, were very encouraging. Again it has been demonstrated that with an appropriate UV dose greater than the 30-40 mJ/cm^2 this system was supplying, the levels of taint producing *Alicyclobacillus* would likely be reduced below the quoted detection sensitivity of the SMX 012A-250 test. Radom attempts to enumerate overall TAB counts on the reaming amounts of sample yielded colony reduction in the 60 to 70% range.

Conclusions

Due to the confirmed TAB presence and the desire on the part of ASR to extend its product line to produce medium invert sugar syrup for use by those beverage companies producing pasteurized acidic beverage business, it became necessary to investigate methods to mitigate or eliminate the presence of these organisms. In the end, UV irradiation was chosen as the most viable and likely alternative process to be studied. Both a pilot low pressure lamp and a commercially placed medium pressure system were evaluated for their effectiveness in eliminating both *Alicyclobacillus* and guaiacol producing *A. acidoterrestris*, from 74-75 brix sugar syrup at 71-77°C with a %UVT in the area of 32-40% @253.7 nm.

In the case of the low pressure system multiple trials were conducted at different syrup UV dosage levels, calculated based on clean water dosages applied from 74 to 545 mJ/cm^2. In each case overall TAB colony reduction increased with increasing UV dose. A D10 dose (based on clean water UVT) was estimated to be 312mJ/cm^2. However, even the lower dosed syrup with overall TAB colony reductions in the area of 50% on average yielded negative results (<1cfu/50g sample) for the presence of *A. acidoterrestris*. Two explanations for this result were considered: 1) a partial UV elimination of an already very low original population of *A. acidoterrestris* affecting the test detection occurred or, 2) a possible increased sensitivity of organism damage of the *A. acidoterrestris* species resulted when compared to other TAB species.

The results for the medium pressure UV lamp trials were comparable with the vast majority of irradiated samples yielding undetectable results for the presence of *A. acidoterrestris*. Overall, the percent colony eliminations were in the area of 60-70%. However, taking into account the system was designed for syrup of 40 %UVT, the few positive results yielded for *A. acidoterrestris* on effluent samples and the less than 90% overall *Alicyclobacillus* elimination suggests that the design dosage will need to be significantly higher than the current design to assure its' effectiveness when compared to the low pressure system.

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